L Number	Hits	Search Text	DB	Time stamp
1	1578	(210/656).CCLS.	USPAT; US-PGPUB	2002/09/11 09:46
2	29569	mass adj spectro$	USPAT; US-PGPUB	2002/09/11 09:47
3	167	((210/656).CCLS.) and (mass adj spectro$)	USPAT; US-PGPUB	2002/09/11 09:47
4	13770	protein adj protein	USPAT; US-PGPUB	2002/09/11 09:47
5	6	(((210/656).CCLS.) and (mass adj spectro$)) and (protein adj protein)	USPAT; US-PGPUB	2002/09/11 10:03
6	750	(250/281).CCLS.	USPAT; US-PGPUB	2002/09/11 10:04
7	17890	affinity adj chromatography	USPAT; US-PGPUB	2002/09/11 10:05
8	2	((250/281).CCLS.) and (affinity adj chromatography)	USPAT; US-PGPUB	2002/09/11 10:19
9	639	(436/173).CCLS.	USPAT; US-PGPUB	2002/09/11 10:20
10	196	(mass adj spectro$) and ((436/173).CCLS.)	USPAT; US-PGPUB	2002/09/11 10:41
11	629	(436/174).CCLS.	USPAT; US-PGPUB	2002/09/11 10:42
12	86	(mass adj spectro$) and ((436/174).CCLS.)	USPAT; US-PGPUB	2002/09/11 10:42
13	51329	electrophore$	USPAT; US-PGPUB	2002/09/11 10:42
14	26	((mass adj spectro$) and ((436/174).CCLS.)) and electrophore$	USPAT; US-PGPUB	2002/09/11 10:49
15	20	(((mass adj spectro$) and ((436/174).CCLS.)) and electrophore$) and chromatography	USPAT; US-PGPUB	2002/09/11 10:49
16	5	(affinity adj chromatography) and (((mass adj spectro$) and ((436/174).CCLS.)) and electrophore$)	USPAT; US-PGPUB	2002/09/11 10:57
17	433	microcolumn	USPAT; US-PGPUB	2002/09/11 10:57
18	134	(mass adj spectro$) and microcolumn	USPAT; US-PGPUB	2002/09/11 10:59
19	82	electrophore$ and ((mass adj spectro$) and microcolumn)	USPAT; US-PGPUB	2002/09/11 11:11
20	3	(protein adj protein) and (electrophore$ and ((mass adj spectro$) and microcolumn))	USPAT; US-PGPUB	2002/09/11 10:59
21	0	immunoaffinity adj colomn	USPAT; US-PGPUB	2002/09/11 11:11
22	1655	immunoaffinity adj column	USPAT; US-PGPUB	2002/09/11 11:11
23	0	(electrophore$ and ((mass adj spectro$) and microcolumn)) and (immunoaffinity adj column)	USPAT; US-PGPUB	2002/09/11 11:11

DIG / 635, 656, 158.2

436 / 161, 173, 174, 178, 63

(FILE 'HOME' ENTERED AT 12:37:04 ON 11 SEP 2002)

FILE 'CAPLUS, MEDLINE, BIOSIS, CA, SCISEARCH, EMBASE' ENTERED AT 12:37:13 ON 11 SEP 2002

L1	5567	S	MICROCOLUMN
L2	18857	S	AFFINITY (W) COLUMN
L3	674761	S	MASS (W) SPECTRO?
L4	662	S	L1 AND L3
L5	270		DUPLICATE REM L4 (392 DUPLICATES REMOVED)
L6	934944	S	ELECTROPHORESIS
L7	44	S	L5 AND L6
L8	44		DUPLICATE REM L7 (0 DUPLICATES REMOVED)
L9	1124070	S	AFFINITY
L10	3	S	L8 AND L9
L11	16	S	AFFINITY (W) MICROCOLUMN
L12	2	S	L3 AND L11
L13	92730	S	AFFINITY (W) CHROMATOGRAPHY
L14	2086	S	L3 AND L13
L15	565	S	L6 AND L14
L16	1479	S	L3 (S) L13
L17	356	S	L16 AND L15
L18	158		DUPLICATE REM L17 (198 DUPLICATES REMOVED)
L19	309728	S	EXTRACT
L20	11	S	L18 AND L19

ANSWER 3 OF 3 SCISEARCH COPYRIGHT 2002 ISI (R)
AN 96:602988 SCISEARCH
GA The Genuine Article (R) Number: BG02E
TI **MICROCOLUMN** LIQUID-CHROMATOGRAPHY IN BIOCHEMICAL-ANALYSIS
AU NOVOTNY M V (Reprint)
CS INDIANA UNIV, DEPT CHEM, BLOOMINGTON, IN, 47405 (Reprint)
CYA USA
SO METHODS IN ENZYMOLOGY, (1996) Vol. 270, Part A, pp. 101-133.
ISSN: 0076-6879.
DT General Review; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 106

ANSWER 14 OF 44 CAPLUS COPYRIGHT 2002 ACS

TI Electrospray ionization time-of-flight **mass spectrometric** detection for fast liquid phase separations

AB This paper describes the design and performance of a new time-of-flight **mass spectrometric** (TOFMS) detector for liq. chromatog. and capillary **electrophoresis**. Emphasis is placed on speed and sensitivity. TOFMS is developing exponentially compared to all other MS techniques. The attributes of TOFMS, such as unlimited mass range, high ion transmission efficiency, high duty cycle, and simplicity, fortunately complement the high speed and sensitivity characteristics, and qualify the TOFMS as one of the most powerful detectors for **microcolumn** sepns.

SO American Laboratory (Shelton, Connecticut) (2000), 32(3), 110, 112-114, 116-119
CODEN: ALBYBL; ISSN: 0044-7749

AU Lazar, Iulia M.; Lee, Edgar D.; Sin, Joseph C. H.; Rockwood, Alan L.; Onuska, Kenneth D.; Lee, Milton L.

L8 ANSWER 25 OF 44 MEDLINE

TI Capillary column chromatography improves sample preparation for **mass spectrometric** analysis: complete characterization of human alpha-enolase from two-dimensional gels following in situ proteolytic digestion.

AB Two-dimensional polyacrylamide gel **electrophoresis** (2-DE) in combination with **mass spectrometry** is an extremely powerful tool for characterizing complex mixtures of proteins. In many cases, the success of this approach relies upon the ability to recover peptides at high concentrations and free of interfering artifacts from in-gel and/or on-membrane enzymatic digests. In previous studies, we demonstrated that capillary or **microcolumn** (< 350 microm ID) reversed-phase high performance liquid chromatography (RP-HPLC) is a powerful microseparation technique for proteins and peptides (Moritz, R. L. and Simpson, R. J., J. Chromatogr. 1992, 599, 119-130). Here we evaluate various capillary column RP-HPLC/**mass spectrometric** approaches for identifying and characterizing 2-DE resolved proteins. For these studies, stable and efficient 0.20 mm and 0.32 mm internal diameter (ID) fused-silica columns with hydrophilic polyvinylidene difluoride (PVDF) frits were fabricated and slurry packed with 7 microm spherical, 300 A pore size, C8 bonded phase silica particles. We show that capillary column chromatography is a rapid and efficient desalting/concentrating (ON/OFF) technique for sample cleanup prior to protein identification by peptide-mass fingerprinting using matrix-assisted laser desorption ionization (MALDI)-time-of-flight **mass spectrometry**. While marginally more peptide mass information can be obtained by stepped elution of the peptide mixture with increasing concentrations of organic solvent, best results were obtained by fractionation of the peptide mixture using a linear 60 min gradient. One salient feature of this study was the observation that, in contrast to the stepped elution and gradient approaches, the ionization of peptide T1 (m/z 2402.2 SGETEDTFIADLVV(PeCys)TGQIK) was almost completely suppressed using the ON/OFF approach. Maximal amino acid sequence coverage, a necessary prerequisite for complete characterization of a protein, was accomplished using a capillary column (0.2 mm ID) directly coupled with an electrospray ionization (ESI) ion-trap tandem **mass spectrometer**. For example, from an in situ tryptic digest of alpha-enolase isolated by 2-DE from the human breast carcinoma cell line MDA-MB231, 71% of the amino acid sequence was obtained. In addition to identifying two possible N-terminal acetylated alpha-enolase variants, Asn153Asp and Ile152Asp/Asn153Ile, the tandem **mass spectrometric** data revealed the presence of a number of process-induced modifications of alpha-enolase such as methionine oxidation and cysteine amidoethylation.

SO ELECTROPHORESIS, (1998 May) 19 (6) 946-55.
Journal code: 8204476. ISSN: 0173-0835.

AU Reid G E; Rasmussen R K; Dorow D S; Simpson R J

L8 ANSWER 27 OF 44 CAPLUS COPYRIGHT 2002 ACS

TI Time-of-flight **mass spectrometry** detection for **microcolumn** separations.

AB The development of a new generation of sensitive and high speed **mass spectrometer** detectors, the time-of-flight **mass spectrometer** (TOFMS), has been fueled by rapid progress being made in the fields of chromatog. and capillary **electrophoresis**. This paper reports on investigations of interfacing **microcolumn** sepns. with TOFMS using atm. pressure ionization sources. The focus of the research was the development of a TOFMS and its evaluation as a detector for fast sepns. The performance and limitations of the instrument will be discussed, and relevant examples will be presented. High-speed spectral acquisition, high spectral storage rate, and attomole sensitivity have been achieved. Applications which will be described include pharmaceuticals, environmental pollutants, and peptides.

SO Book of Abstracts, 216th ACS National Meeting, Boston, August 23-27 (1998), ANYL-219 Publisher: American Chemical Society, Washington, D. C. CODEN: 66KYA2

AU Lazar, I. M.; Sin, C. H.; Rockwood, A. L.; Lee, E. D.; Collins, D. C.; Xin, B.; Lippert, A. J.; Chen, S.; Lee, M. L.

(FILE 'HOME' ENTERED AT 09:17:48 ON 12 SEP 2002)

FILE 'CAPLUS, MEDLINE, BIOSIS, CA, SCISEARCH, EMBASE' ENTERED AT 09:17:54 ON 12 SEP 2002

L1	14355	S	INTERACTING (W) PROTEIN#
L2	675010	S	MASS (W) SPECTRO?
L3	117	S	L1 (S) L2
L4	35		DUPLICATE REM L3 (82 DUPLICATES REMOVED)
L5	1652123	S	CHROMATOGRAPHY
L6	9	S	L4 AND L5
L7	935134	S	ELECTROPHORESIS
L8	2	S	L6 AND L7

FILE 'CAPLUS, MEDLINE, BIOSIS, CA, SCISEARCH, EMBASE' ENTERED AT 13:34:02 ON 11 SEP 2002

L1	17205	S	INTERACT? (W) PROTEIN#
L2	92730	S	AFFINITY (W) CHROMATOGRAPHY
L3	166	S	L1 AND L2
L4	63		DUPLICATE REM L3 (103 DUPLICATES REMOVED)
L5	674761	S	MASS (W) SPECTRO?
L6	8	S	L4 AND L5
L7	199	S	L1 AND L5
L8	934944	S	ELECTROPHORESIS

ANSWER 1 OF 2 CAPLUS COPYRIGHT 2002 ACS

TI Methods for systematic identification of protein - protein interactions and other properties

AB The invention concerns a method for identifying protein-protein interactions. The **interacting proteins** may be isolated by affinity chromatog. and may be identified and characterized by **mass spectrometry**. The invention in part allows for the high throughput anal. of protein-protein interactions that lends itself to automation.

SO PCT Int. Appl., 78 pp.
CODEN: PIXXD2

IN Awrey, Donald E.; Greenblatt, Jack

L8 ANSWER 2 OF 2 MEDLINE

TI Overexpression, purification, and characterization of glutaminase-interacting protein, a PDZ-domain protein from human brain.

AB A human brain cDNA clone coding for a novel PDZ-domain protein of 124 amino acids has been previously isolated in our laboratory. The protein was termed GIP (glutaminase-**interacting protein**) because it interacts with the C-terminal region of the human brain glutaminase L. Here we report the heterologous expression of GIP as a histidine-tagged fusion protein in Escherichia coli cells. The induction conditions (temperature and isopropyl beta-d-thiogalactopyranoside concentrations) were optimized in such a way that GIP accounted for about 20% of the total E. coli protein. A simple and rapid procedure for purification was developed, which yielded 17 mg of purified GIP per liter of bacterial cell culture. The apparent molecular mass of the protein by SDS-PAGE was 16 kDa, whereas in native form it was determined to be 28 kDa, which suggests dimer formation. The nature and integrity of the recombinant protein were verified by **mass spectrometry** analysis. The functionality of the GIP protein was tested with an in vitro activity assay: after being pulled down with glutathione S-transferase-glutaminase, GIP was revealed by Western blot using anti-GIP antibodies. Furthermore, the glutaminase activity in crude rat liver extracts was inhibited by the presence of recombinant purified GIP protein.

SO PROTEIN EXPRESSION AND PURIFICATION, (2001 Dec) 23 (3) 411-8.
Journal code: 9101496. ISSN: 1046-5928.

AU Aledo J C; Rosado A; Olalla L; Campos J A; Marquez J

L6 ANSWER 7 OF 9 MEDLINE

TI Affinity-purification and characterization of caveolins from the brain: differential expression of caveolin-1, -2, and -3 in brain endothelial and astroglial cell types.

AB Caveolins 1, 2 and 3 are the principal protein components of caveolae organelles. It has been proposed that caveolae play a vital role in a number of essential cellular functions including signal transduction, lipid metabolism, cellular growth control and apoptotic cell death. Thus, a major focus of caveolae-related research has been the identification of novel caveolins, caveolae-associated proteins and caveolin-**interacting proteins**. However, virtually nothing is known about the expression of caveolins in brain tissue. Here, we report the purification and characterization of caveolins from brain tissue under non-denaturing conditions. As a final step in the purification, we employed immuno-affinity **chromatography** using rabbit polyclonal anti-caveolin IgG and specific elution at alkaline pH. The final purified brain caveolin fractions contained three bands with molecular masses of 52 kDa, 24 kDa and 22 kDa as visualized by silver staining. Sequencing by ion trap **mass spectrometry** directly identified the major 24-kDa component of this hetero-oligomeric complex as caveolin 1. Further immunocyto- and histochemical analyses demonstrated that caveolin 1 was primarily expressed in brain endothelial cells. Caveolins 2 and 3 were also detected in purified caveolin fractions and brain cells. The cellular distribution of caveolin 2 was similar to that of caveolin 1. In striking contrast, caveolin 3 was predominantly expressed in brain astroglial cells. This finding was surprising as our previous studies have suggested that the expression of caveolin 3 is confined to striated (cardiac and skeletal) and smooth muscle cells. Electron-microscopic analysis revealed that astrocytes possess numerous caveolar invaginations of the plasma membrane. Our results provide the first biochemical and histochemical evidence that caveolins 1, 2 and 3 are expressed in brain endothelial and astroglial cells.

6 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2002 ACS

TI Isolation of protein subpopulations undergoing protein-protein interactions

AB A new method is described for isolating and identifying proteins participating in protein-protein interactions in a complex mixt. The method uses a cyanogen bromide-activated Sepharose matrix to isolate proteins that are non-covalently bound to other proteins. Because the proteins are accessible to chem. manipulation, **mass spectrometric** identification of the proteins can yield information on specific classes of **interacting proteins**, such as calcium-dependent or substrate-dependent protein interactions. This permits selection of a subpopulation of proteins from a complex mixt. on the basis of specified interaction criteria. The new method has the advantage of screening the entire proteome simultaneously, unlike the two-hybrid system or phage display, which can only detect proteins binding to a single bait protein at a time. The method was tested by selecting rat brain ext. for proteins exhibiting calcium-dependent protein interactions. Of 12 proteins identified by mass spectrometry, eight were either known calcium-binding proteins or proteins with known calcium-dependent protein interactions, indicating that the method is capable of enriching a subpopulation of proteins from a complex mixt. on the basis of a specific class of protein interactions. Because only naturally occurring interactions of proteins in their native state are obsd., this method will have wide applicability to studies of protein interactions in tissue samples and autopsy specimens, for screening for perturbations of protein-protein interactions by signaling mols., pharmacol. agents or toxins, and screening for differences between cancerous and untransformed cells.

SO Molecular and Cellular Proteomics (2002), 1(3), 253-259
CODEN: MCPOBS; ISSN: 1535-9476

AU Nelson, Thomas J.; Backlund, Peter S., Jr.; Yergey, Alfred L.; Alkon, Daniel L.